감염성 비만 연구의 과거, 현재, 그리고 미래

The Yesterday, Today, and Tomorrow of Pathogen-induced Obesity

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요 약

미국 의학협회에서는 최근 비만을 질병으로 규정하였다. 비만은 유전적, 사회적, 환경적, 생리적 원인 등 매우 다양하다. 이러한 원인들 중 특정 병원균의 감염에 의해 비만이 되는 것을 감염성 비만(infectobesity)이라고 부른다. 특히 사람 아데노바이러스 36(Ad36)은 동물 및 사람의 지방 증가와 연관이 있는 것으로 알려져 있다. 흥미로운 사실은 이 바이러스에 의해 유도되는 비만은, 고지혈증 유도 비만과는 달리, 혈청 트리글리세리드, 콜레스테롤, 인슐린 등이 감소하는 등 혈당 조절이 개선되는 현상을 보인다는 것이다. 한국, 미국, 이탈리아 등에서 발표된 논문들에 의하면 Ad36의 항체를 가진 사람의 비만이 비만과 연관되어 있다고 한다. 항체 양성인 그룹은 지방 및 체질량지수(body mass index, BMI)의 증가를 보여주나, 혈청 지방 및 인슐린 레벨은 감소하고 있음을 보여준다. 이러한 현상은 아마도 바이러스 감염에 의한 염증, 미토콘드리아 활성, 포도당 흡수촉진 등의 영향을 받은 것으로 볼 수 있다. 특히, 바이러스 유전자인 E4orf1만으로도 지방생성 및 포도당 흡수촉진이 증가하는 현상을 보여주고 있다. 따라서 E4orf1이 고인슐린혈증의 치료 후보물질 타겟이 될 수 있을 것이다. 종합해보면, Ad36 및 E4orf1이 비만에 의한 대사질환의 치료제로 사용될 가능성이 있다고 할 수 있다.

중심단어: 감염성 비만, 사람 아데노바이러스 36, E4orf1, 염증, 염증 조절

ABSTRACT

The American Medical Association officially declared obesity a disease. However, obesity is caused by genetic, social, environmental, and physiological factors. Among the many factors, infection by some pathogens has a significant impact on obesity and has been called infectobesity. In particular, human adenovirus 36 (Ad36) increases adipose tissue in animals and body fat in humans. Interestingly, Ad36-induced obesity paradoxically improves glycemic control by decreasing serum triglycerides, cholesterol, and insulin, in contrast to high-fat diet obesity. Some epidemiological studies in Korea, the US, and Italy have demonstrated that Ad36 infection is associated with human obesity. The virus-infected subjects in those studies showed increased body fat and body mass index but decreased serum lipid and insulin levels. This phenomenon may be affected by inflammation, mitochondrial activity, and glucose uptake. Moreover the Ad36 gene, E4orf1, also increases adipogenesis and improves glucose uptake. Therefore, E4orf1 may be a template for a therapeutic agent to treat hyperinsulinemia. Thus, Ad36 and E4orf1 are crucial therapeutic agents to treat obesity-related metabolic diseases.

Key words: Infectobesity, Human adenovirus 36, E4orf1, Inflammation, Glycemic control
Definition of infectobesity

The increasing prevalence of obesity and diabetes has become a major public health problem. The World Health Organization estimates that 300 million adults worldwide are obese. Moreover, the American Medical Association recently classified obesity as a disease. The general pathophysiological cause of obesity is a disturbance in the energy balance by excess energy intake. The balance between energy intake and expenditure is influenced by complex genetic, social, and environmental factors (psychology, smoking, exercise, stress, and diet). It is generally accepted that obesity is a risk factor for metabolic disease at the population level. However, how obesity status progresses to metabolic syndrome is unclear.

Although various genetic, social, and cultural factors trigger obesity, infection by pathogens is also an obesity trigger. Ten pathogens have been shown to cause increasing adiposity in animals over the past two decades (Table 1). Based on these reviews, pathogens can induce adiposity and associated metabolic diseases. Thus, this concept is called “infectobesity.” Some pathogens increase adiposity by impairing the central nervous system. The canine distemper virus was the first reported obesity-inducing virus. Rous-associated virus-7 causes obesity and hyperlipidaemia in chickens. Borna disease virus causes adiposity in rats, and scrapie agents induce

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<thead>
<tr>
<th>Table 1. The pathogens induce adiposity in animals and human</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristics of adiposity</strong></td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>Scrapie agents</td>
</tr>
<tr>
<td>Canine distemper Virus (CDV)</td>
</tr>
<tr>
<td>Rous-associated Virus type 7 (RAV-7)</td>
</tr>
<tr>
<td>Avian adenovirus (SMAM-1)</td>
</tr>
<tr>
<td>Borna disease virus (BDV)</td>
</tr>
<tr>
<td>Chlamydia Pneumonia Human adenovirus 36 (Ad36)</td>
</tr>
<tr>
<td>Human adenovirus 37 (Ad37)</td>
</tr>
<tr>
<td>Human adenovirus 5 (Ad5)</td>
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<tr>
<td>Gut microbiota</td>
</tr>
</tbody>
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MCH, melanin-concentrating hormone; NPY, neuropeptide Y; GLUT4, glucose transporter type 4; HOMA, homoeostatic model assessment; p.i., post infection. This table was quoted and modified from the studies.
adiposity in mice. Microbiota such as bacteria, archaea, viruses, and eukaryotes have been linked with human obesity.\textsuperscript{8,9} In addition, four adenoviruses increase adiposity. SMAM-1, an avian adenovirus, is associated with human obesity and animal adiposity.\textsuperscript{8,9} Human adenovirus (Ad) 5, Ad36, and Ad37 causes adiposity in animals.\textsuperscript{8,9} In particular, Ad36 is associated with human obesity and increased body mass index (BMI) and waist circumstance.\textsuperscript{8-10} Moreover, Ad36 also increases adiposity in animals such as chickens, mice, rats, and monkeys.

Epidemiological proof of the relationship between obesity and human adenovirus 36

Adenoviruses easily infect via respiratory, venereal, and fecal-oral routes.\textsuperscript{10} Ad36 was first isolated from feces of diabetic girls.\textsuperscript{9} This virus is included in the D subgroup of human adenoviruses that cause respiratory diseases, conjunctivitis, and tonsillitis.\textsuperscript{6} Ad36 is antigenetically distinctive from other adenoviruses and does not show serological cross-reactivity with them.\textsuperscript{11} Among human adenoviruses, Ad36 is strongly associated with human obesity in Korea, Italy, and the US (Fig. 1).\textsuperscript{12} In Korea, 29% of obese subjects are Ad36 sero-positive, but only 14% of non-obese school children have Ad36 antibodies.\textsuperscript{13} Therefore, Ad36 sero-positivity is greater in obese than that in non-obese subjects. In addition, Ad36 is also associated with overweight in Korean adults.\textsuperscript{14} Ad36 sero-positive subjects could be a marker of a clinical-metabolic profile preceding obesity in Italy.\textsuperscript{15} Approximately 65% of Ad36 sero-positive subjects are obese and 33% of non-obese subjects have Ad36 antibodies. Ad36 sero-positive subjects are significantly older and have a larger BMI and waist/hip ratio. Approximately 30% of obese US adults have Ad36 antibodies, whereas 11% of non-obese subjects have the antibodies.\textsuperscript{16} The sero-positive subjects have higher BMIs and body fat than those of sero-negative subjects. About 22% of obese US children are positive for the Ad36 antibody, and 7% of non-obese are positive.\textsuperscript{17} In addition, sero-positive subjects have significantly higher anthropometric measures including body weight, BMI, and waist/height ratio. Ad36 is associated with obesity after 10 years.\textsuperscript{18}

However, some specific cohorts do not show a relationship between Ad36 infection and obesity. About 34% of the obese and 39% of the lean subjects in the US military have Ad36 antibodies.\textsuperscript{19} However, this cohort has a different background compared to other cohorts, as soldiers exercise regularly. For this reason, we cannot say that the virus is not associated with obesity. In addition, studies in Belgium and the Netherlands show that Ad36 does not play a direct role in increased BMI and obesity\textsuperscript{20}, as only 8% of Belgians have Ad36 antibodies. Therefore, although many epidemiological studies have demonstrated that Ad36 infection is strongly associated with obesity, more longitudinal and cross-sectional epidemiological studies based on many races, ages, and countries are required.

Biological characteristics of human adenovirus 36

Ad36 infection can increase adiposity in animals but paradoxically improves glycemic control.\textsuperscript{21,22} The infection increases body weight, fat, and BMI, but decreases total cholesterol, triglycerides, free fatty acids,
and insulin in fasting human serum.\textsuperscript{15,16,18} Ad36 directly enhances adipocyte expansion and increases the number of adipocytes, and the expanded adipocytes can more take more glucose from the blood.\textsuperscript{21} Moreover, Ad36 induces inflammation in adipose tissues, as shown by increases in M1 macrophage and pro-inflammatory adipokines, and this inflammation may be required to develop adiposity. MCP-1 knockout mice are not obese and develop improved glycemic control after Ad36 infection.\textsuperscript{24} Thus, we focused on how Ad36 can improve glycemic control and increase adiposity after viral infection in this review.

1. Adipose tissue expandability by Ad36 infection

Ad36 increases adiposity such as body weight and fat in animals.\textsuperscript{21-24} In an initial experiment, chickens were injected with Ad36 and sacrificed 5 weeks post injection.\textsuperscript{25} Visceral and total body fat of the Ad36-injected group were significantly greater compared to those in the control group, but serum triglycerides and cholesterol were lower than those in the control group. When the chickens were infected by intranasal and intraperitoneal injections, they showed decreased serum cholesterol following the intranasal Ad36 infection, but serum cholesterol and triglycerides did not decrease following the intraperitoneal injection.\textsuperscript{21} Body weight of the mice was 9\% greater, body fat was 35\% greater, and visceral fat was 67\% greater in Ad36-injected mice at 22 weeks after injection. However, Ad36-injected mice showed a decrease in serum cholesterol and triglycerides compared to those in a control group. Ad36 induced a three-fold weight gain and fat gain in male rhesus monkeys at 36 months post injection.\textsuperscript{26} However, Ad36-injected monkeys have decreased serum cholesterol compared to that in the control group. Ad36 transmissibility was investigated in other experiments.\textsuperscript{25} When uninfected chickens are housed with Ad36-infected chickens, the uninfected chickens became infected through the virus-injected chickens. When the uninfected chickens were injected with blood from Ad36-infected chickens, all chicken showed increased antibody titers to Ad36 and increased visceral and total body fat.\textsuperscript{25} Thus, Ad36 can increase adiposity but decrease serum cholesterol and triglycerides.

Trovato et al\textsuperscript{27} investigated the correlation between Ad36 and human non-alcoholic fatty acid liver disease (NAFLD). It is well known that NAFLD is associated with obesity and insulin resistance.\textsuperscript{28,29} However, although Ad36 seropositivity is associated with greater adiposity, it was not associated with a significant difference in insulin resistance in patients with NAFLD. In contrast, Ad36 seropositivity is associated with a lower risk of NAFLD and a bright liver.\textsuperscript{27} This indicates that Ad36 infection may help improve human metabolic disease. Ad36-seropositive Korean school children have increased BMI and body fat.\textsuperscript{13} However, they also showed increased total cholesterol, triglycerides, and LDL-cholesterol. Ad36 decreases serum lipid and insulin in Italian and US adults.\textsuperscript{15,16} Ad36 increases body fat and BMI in both adults and children, but the virus decreases serum lipid and insulin in adults\textsuperscript{15,16}, except in Korean children.\textsuperscript{13} Therefore, the adipose tissue can reduce excess glucose in blood through a rise in glucose uptake, so blood levels of glucose and insulin are reduced. This phenomenon is called adipose tissue expandability and may be the cause for improved glycemic control in subjects with Ad36.

2. Mitochondrial activity following Ad36 infection

The most important role of mitochondria is to produce energy through respiration, and to regulate cellular metabolism.\textsuperscript{30} Thus, mitochondria produce ATP, which oxidizes glucose, pyruvate, and NADH. Therefore, damage and dysfunction in mitochondria influences cell metabolism and human disease.\textsuperscript{31} Dysfunction of mitochondria may cause some diseases such as Alzheimer’s disease, Kearns-Sayre syndrome, cardiovascular diseases, and diabetes.\textsuperscript{32}

Although Ad36 increases adiposity, the virus improves hepatic steatosis and glycemic control, which means decreased serum lipids and insulin. According to previous data\textsuperscript{21,25,26}, Ad36 decreases serum cholesterol and triglycerides in chickens, mice, and monkeys. One of reasons for this effect is that Ad36 enhances liver mitochondrial activity. We confirmed mitochondrial activity in the liver at 3 months after infecting mice with Ad36 (unpublished data). Cytochrome c activity and integrity of mitochondria improves in the liver of Ad36-infected mice. Moreover, Ad36 increases mitochondrial transcription factors such as mtDNA, PGC-1, NRF-1, and UCP-1 in the liver. The number of liver mitochondria increases compared to that in a control
group. Ad36 can reduce hepatic lipid accumulation and liver weight, and significantly increases glycogen content even in high-fat diet mice. Moreover E4orf1, an Ad36-specific protein, increases de novo lipogenesis, palmitate oxidation, and lipid export in expressed-HepG2 cells. In addition, E4orf1 significantly decreases glucose output and glucose transporter 2 (GLUT2) under basal and gluconeogenic conditions. This result indicates that Ad36 and E4orf1 improves hepatic glucose and lipid metabolism. Ad36 can also systemically improve serum lipid and insulin levels. However, Ad36 reduced cytochrome c activity, mitochondrial integrity, and size of the mitochondria in subcutaneous fat and muscle (unpublished data). Therefore, Ad36 acts differently in each organ, so mitochondrial activity may be affected by other phenomena. Taken together, Ad36-increased mitochondrial activity in the liver may be one of the ways to improve glycemic control after virus infection.

3. Inflammation in adipose tissues by Ad36 infection

Obesity increases body fat, which remodels adipose tissues through the actions of cytokines and angiogenesis and then generates inflammation. Ad36 also increases body weight and body fat in animals, and the virus causes inflammation of adipose tissue. Ad36 increases tumor necrosis factor (TNF)-α expression 2.5-fold compared with that in mock-infected mice. Moreover, Ad36 significantly upregulates mRNA levels of chemotactic protein (MCP) 1 and CD68. Ad36 infection increases epidermal fat in C57BL/6 mice, and upregulates pro-inflammatory cytokines such as MCP 1 and TNF-α in fat. These cytokines impair the downregulation of insulin receptor signaling, and induce infiltration of macrophages and other immune cells. Ad36 infection increases infiltration of macrophages (F4/80) and induces differentiation of M1 macrophages that cause pro-inflammatory states in adipose tissue. We found that Ad36 induced acute inflammation 7 days after infection, whereas a high-fat diet did not induce acute inflammation (7 days after feeding) in mouse epidermal fat tissue (unpublished data).

Adipose tissues can be remodeled by inflammation and angiogenesis, and remodeling adipose tissue continually maintains the obesity state. In particular, MCP-1-induced protein (MCPIP) induces adipogenesis in 3T3L1 cells. MCPIP expression increases the CCAAT/enhancer-binding proteins (C/EBP) family of adipogenesis transcription factors. Ad36 infection does not increase body fat in MCP1 knockout mice but decreases pro-inflammatory cytokines. Moreover, infiltration of macrophages decreases in epidermal fats of MCP1 knockout mice despite Ad36 infection. Interestingly, MCP1 knockout mice do not show improved glycemic control after virus infection. Taken together, these data indicate that Ad36 infection induces inflammatory states in fats, and that this inflammation may be required to maintain obesity and improve glycemic control after virus infection.

4. Exercise effect by Ad36 infection

Obesity increases adipose tissue mass and inflammation in fat. Most people exercise to decrease obesity, because exercise effectively reduces body fat, lipid accumulation in tissues, and improves serum hyperlipidemia. Exercise also increase mitochondrial activity. Exercise stimulates PGC-1α in the nucleus, and PGC-1α acts as transcriptional factor to upregulate NRF-1. NRF-1 is related to fat metabolism, which increases mtDNA to promote mitochondria activity. Thus, exercise increases mitochondrial activity in liver, muscle, and subcutaneous fat even in mice fed a high-fat diet. Ad36 infection also significantly increased mitochondria activity in organs of both non-exercised and excised mice (unpublished data). Ad36 infection and exercise synergistically increase liver activity, and improve glycemic control. Ad36-infected mice have decreased serum glucose, cholesterol, and insulin compared to those in mock and negative virus-infected mice (unpublished data). In contrast to the viral infection effect on the liver, Ad36 infection impairs mitochondrial activity and decreases the size of mitochondria, but increases inflammation in fat and muscle tissue even in exercised mice. Therefore, Ad36 infection may prevent the effects of exercise, in that reduction of body weight, due to Ad36-induced adiposity via increase of glucose uptake and inflammation, whereas synergistically improve glycemic control with exercise.

Ad36 signaling mechanisms

1. Adipogenesis by Ad36 infection

Ad36 infection increases glucose uptake and lipid accumulation. Ad36 induces adipogenesis in fat cells with
or without a differentiation cocktail. When fat cells are infected with Ad36, the virus up-regulates C/EBPα, aP2, and PPARγ2, which are transcriptional factors that regulate adipogenesis. Ad36 triggers the RAS/PI3-kinase (PI3K)/Akt pathway for adipogenesis, and increases Akt phosphorylation and activity. Ad36 infection alters cell morphology such as rounded shape, and increases lipid droplets in cells. In particular, the virus induces differentiation in human adipose-derived stem cells, skeletal muscle cells, and human mesenchymal stem cells. Ad2, as a negative control virus did not change adipogenesis genes in microarray data. However, Ad36 upregulated genes related to adipocyte differentiation. The virus is crucial in some pathways such as mitochondrial and PPARγ pathways. Interestingly, Ad36 increases adiponectin abundance and glucose uptake despite impaired or absent PPARγ expressing cells. Therefore, Ad36 can affect without PPARγ induction associated with thiazolidinediones actions and relate to other glycemic control pathways for.

2. Glucose uptake by Ad36 infection

Ad36 infection increases the fat cell size in mice via increased glucose uptake into adipocytes. Previous studies showed that Ad36 induces glucose uptake in preadipocytes, adipocytes, and skeletal muscle cells by distal insulin signaling (Fig. 2). Insulin triggers the insulin receptor and phosphorylated insulin receptor substrates (IRS), and induces the PI3K pathway in most insulin signaling pathways. However, Ad36 blocks IRS phosphorylation, but activates RAS signaling and the PI3K pathway, and then induces transport of glucose transporters such as GLUT4 into the plasma cell membrane for glucose uptake. Thus, the virus induces increased RAS and GLUT4 abundance in skeletal muscle and fat and Akt and AMPK phosphorylation. Therefore, Ad36 induces glucose uptake though the distal insulin signaling pathway. These observations indicate that Ad36-triggered GLUT4 transport is not dependent on the proximal insulin signaling pathway and suggest the possibility to develop a novel drug for insulin-resistant patients.

The characteristics and application of E4orf1

E4orf1 (the first open reading frame of Ad36 early gene 4) is a viral gene involved in regulating transcription of viral DNA. The gene is a 17 kDa, 125 amino acid protein, and has a PDZ-binding motif (PBM) that is linked with scaffolding between PDZ regions and other proteins. The E4orf1 of Ad36 has similarity compared to other human adenoviruses. Ad30 has 98% similarity, Ad9 has 92% similarity, and Ad8 has 88% similarity compared to the E4orf1 region of Ad36 (Table 2).
E4orf1 expression triggered glucose uptake and lipid accumulation as with Ad36 infection. When E4orf1 is overexpressed in 3T3L1 cells, the gene up-regulated C/EBPα and PPARγ, which are adipogenesis transcriptional regulatory factors.\(^{51}\) E4orf1 has a differentiation effect due to its C-terminal PDZ-binding domain. The Ad9 virus triggers PI3K/Akt adipogenesis pathway when PBM interacts with the Dlg1 protein. However, E4orf1 without the PDZ domain does not increase Akt phosphorylation. Based on these results, E4orf1 of Ad36 solely increases adipogenesis in 3T3L1 cells through the PI3K/Akt pathway, and the PDZ-binding domain is essential for adipogenesis.

Moreover, Ad36 increases glucose uptake in adipocytes and skeletal muscle cells, but deleting E4orf1 decreases glucose uptake despite Ad36 infection.\(^{49}\) In addition, E4orf1 overexpression also induces glucose uptake in preadipocytes, adipocytes, and skeletal muscle cells by distal insulin signaling.\(^{51}\) E4orf1 activates the total RAS and PI3K/Akt pathways and promotes translocation of GLUT1 and 4 into plasma membrane. Dhurandhar et al\(^{51}\) also showed that E4orf1 requires PBM to increase glucose uptake by RAS activation. E4orf1 in HepG2 cells and primary hepatocytes suppresses glucose output by the GLUT2 transporter, and improves hepatic insulin resistance and lipid accumulation in cells.\(^{33}\) E4orf1 increases RAS signaling, but decreases GLUT2 in HepG2 cells. In addition, the agent increases complete palmitate oxidation, but not the ratio of acid soluble metabolites (incomplete) to CO₂ (complete). E4orf1 decreases phosphorylated sterol regulatory element-binding protein-1c, which is a for de novo lipogenesis regulatory factor in the liver, and activates ApoB secretion. Lipid export is related to ApoB-containing lipoprotein secretion in hepatocytes. Therefore, decreasing hepatic glucose output by E4orf1 is an important for improving glycemic control, and E4orf1 reduces lipogenesis in hepatocytes.

The prospects for Ad36 and E4orf1

According to previous studies and unpublished data, Ad36 is associated with the induction of inflammation and adiposity. Inflammation may be required to increase adiposity, and E4orf1 is the trigger responsible for glucose uptake following Ad36 infection. However, the cellular and immunological mechanisms of Ad36-induced obesity are unknown. In this review, we mentioned “adipose tissue expandability”, “mitochondrial activity”, and “inflammation” of adipose tissue. Ad36 and its gene, E4orf1, increase adipose tissue in animals and induce adipocyte differentiation. Thus, Ad36 infection, particularly E4orf1 among viral genes, can accelerate hyperplasia and hypertrophy of adipocytes (Fig. 3).

Ad36 and E4orf1 improve glycemic control by increasing glucose uptake in expanded adipose tissue. However, the pathways involved in the increased of adipose tissue mass must be identified. We suggest that an Ad36 infection triggers acute inflammation, this inflammation generates inflammatory cytokines such as MCP1, and induces infiltration of immune cells into adipose tissue causing chronic inflammation. However, the pathway inducing of inflammatory cytokines is unknown. We suggest that Ad36 infection can be recognized by body’s innate immune system, such as pattern recognition receptors (Toll-like receptors, etc), and that this recognition triggers inflammatory cytokine production. A higher priority is to understand the role of inflammation in virus-induced and high fat diet-induced obesity.
Generally, obesity-induced inflammation triggers metabolic diseases. However, virus-induced inflammation is an essential process to remove infected cells.

Therefore, we think inflammation may have a positive role in obesity except uncontrolled inflammation. This idea is very debatable, but possible.

Interestingly, Ad36 induces inflammation after infection in adipose tissue, whereas E4orf1 may do not. We do not completely understand this opposite phenomenon but Ad36 can affect different signal pathways in infected cells based on viral genes, whereas E4orf1 affects on limited signal pathways.

Taken together, although Ad36 improves glycemic control, the virus itself is very difficult to use as a therapeutic agent. Thus, the function of E4orf1 must be investigated to determine whether it has the same effect regarding glycemic control. We must confirm the function of E4orf1 with regard to glycemic control in vivo. In addition, we must find alternative agents instead of Ad36 and E4orf1 for treating humans. If we understand all of the basic characteristics of Ad36-induced obesity and E4orf1, it would be very promising to develop new therapeutic agents for metabolic diseases, such as diabetes.

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